CANCER

Identification of mismatch repair gene mutations in young patients with colorectal cancer and in patients with multiple tumours associated with hereditary non-polyposis colorectal cancer

R C Niessen, M J W Berends, Y Wu, R H Sijmons, H Hollema, M J L Ligtenberg, H E K de Walle, E G E de Vries, A Karrenbeld, C H C M Buys, A G J van der Zee, R M W Hofstra, J H Kleibeuker

Gut 2006;**55**:1781–1788. doi: 10.1136/gut.2005.090159

Additional table is available online at http://gutjnl.com/supplemental

See end of article for authors' affiliations

Correspondence to: J H Kleibeuker, Department of Gastroenterology, University Medical Center Groningen, PO Box 30. 001, 9700 RB Groningen, The Netherlands; j.h.kleibeuker@int.umcg.nl

Revised 30 March 2006
Accepted 4 April 2006
Published Online First 24 April 2006

Background: Patients with early-onset colorectal cancer (CRC) or those with multiple tumours associated with hereditary non-polyposis colorectal cancer (HNPCC) raise suspicion of the presence of germline DNA mismatch repair (MMR) gene mutations.
Aim: To analyse the value of family history, microsatellite instability (MSI) analysis and MMR protein staining in the tumour to predict the presence of an MMR gene mutation in such patients.
Methods: In 281 patients diagnosed with CRC before the age of 50 years or with CRC and at least one additional HNPCC-associated cancer, germline mutation analysis in *MLH1*, *MSH2* and *MSH6* was carried out with denaturing gradient gel electrophoresis and multiplex ligation-dependent probe amplification. MSI analysis with five consensus markers and MMR protein staining for MLH1, MSH2 and MSH6 were carried out in the tumours.
Results: 25 pathogenic mutations (8 in *MLH1*, 9 in *MSH2* and 8 in *MSH6*) were found. MSI analysis missed three and immunohistochemistry (IHC) missed two mutation carriers. Sensitivities of family history, MSI analysis and IHC for the presence of a mutation were 76%, 82% and 88%, specificities were 64%, 70% and 84%, and positive predictive values were 19%, 23% and 38%, respectively. Multivariate analysis showed the highest odds ratio for IHC (38.3, 95% confidence interval 9.0 to 184). Prevalence of pathogenic germline MMR gene mutations in patients with CRC before the age of 50 years was 6% and in those with ⩾2 HNPCC-associated tumours was 22%. In the second group, no mutation carriers were found among the 29 patients who were diagnosed with their first tumour after the age of 60 years.
Conclusion: Family history, MSI analysis and IHC are indicative parameters to select patients with CRC for MMR gene mutation analysis. The data show that IHC is the best single selection criterion.

Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome is a genetic disorder caused by germline mutations in DNA mismatch repair (MMR) genes, in particular in *MLH1, MSH2* and *MSH6*. HNPCC is characterised by the early onset of gastrointestinal and urogenital cancers, especially colorectal and endometrial cancer. Synchronous and metachronous cancers are common, and multiple colorectal cancers can be observed in 20–40% of people with HNPCC.[1–3]

Originally, HNPCC was a clinical diagnosis, based on the Amsterdam criteria I (ACI).[4] In 1999, the ACI were superseded by the Amsterdam criteria II (ACII), which included extracolonic HNPCC-associated tumours.[5] After the discovery of germline MMR gene mutations as the cause of HNPCC, it soon became clear that these criteria were too stringent, as a large proportion of families carrying germline MMR gene mutations did not fulfil either ACI or ACII. Consequently, several groups formulated new sets of criteria to select patients for mutation analysis in the MMR genes.[6–16] The best known are the Bethesda criteria, published in 1997 and revised in 2004.[17 18] Patients fulfilling one of these criteria should be selected for microsatellite instability (MSI) analysis of their tumours. In the case of MSI, a patient should be offered mutation analysis. Some of the (revised) Bethesda criteria are based on the family history, and others are based on the patient's own history (eg, colorectal cancer (CRC) before the age of 50 years and multiple HNPCC-associated cancers, irrespective of age).[18] As self-reported family history is often not accurate, it is important to recognise potential MMR gene mutation carriers independent of the family history.[19 20]

According to the Bethesda criteria and most of the other sets of criteria, mutation analysis should be restricted to those patients whose tumour shows a high degree of MSI. However, a proportion of cancers of *MSH6* mutation carriers were recently shown not to have MSI.[21 22] Thus, some *MSH6* mutations will be missed using this strategy. Alternatively, MMR protein staining of the tumour could be a valuable and possibly a better method of selecting patients for mutation analysis. Mutation analysis should be offered to those patients whose tumours show absence of staining of one of the MMR proteins.[10 14] An advantage of this method is that absence of staining for one of the MMR proteins directly points towards the putatively mutated gene.

In view of these considerations, we determined the value of MSI analysis and MMR protein staining in tumours for the prediction of the presence of a germline MMR gene mutation

Abbreviations: ACI, Amsterdam criteria I; ACII, Amsterdam criteria II; CRC, colorectal cancer; HNPCC, hereditary non-polyposis colorectal cancer; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability

in a large group of patients fulfilling at least one of the above-mentioned Bethesda criteria, independent of family history—that is, colorectal cancer (CRC) before the age of 50 years or at least two HNPCC-associated cancers. The results were compared with the predictive values of family history. The results should enable formulation of rational guidelines regarding whether or not to offer mutation analysis to a patient with cancer who is at risk of HNPCC.

PATIENTS AND METHODS

Patients

Patients diagnosed with CRC before the age of 50 years and patients with ⩾2 HNPCC-associated cancers, including at least one CRC, irrespective of age and family history, were invited to participate in this study. On the basis of data available at the time of initiation of this study in 1996, colorectal cancer, endometrial cancer, cancer of the small bowel, stomach, pancreas, biliary tract and ovaries, and transitional cell cancer of the pelvis, ureter and bladder were considered to be HNPCC associated. Using the data of the regional cancer registry of the Comprehensive Cancer Center North-Netherlands from 1989 onwards, doctors at the participating hospitals and general practitioners invited patients to take part in the study and referred them to the study coordinator. Patients newly diagnosed from September 1997 onwards were offered participation in the same way, as well as patients who were diagnosed before 1989 with CRC before the age of 50 years or with CRC and at least one other HNPCC-associated cancer, who came to our attention for whatever medical reason. Inclusion ended by December 2000. Patients gave written informed consent after written and verbal pretest counselling. Blood (20 ml) was collected for DNA isolation. A thorough family history for HNPCC-associated cancers was recorded. Clinical data were reviewed. Cancer material was obtained and histology was revised. With permission of the patients involved, medical records of relatives with HNPCC-associated cancers were collected, whenever possible, to verify the nature of the reported cancers. The participating patients were informed about the results of the genetic test, if they so wished. In such instances, they received verbal post-test counselling and a written summary.

Another source of patients was the Department of Clinical Genetics, Groningen, The Netherlands, where patients were referred to and counselled if they were suspected to have HNPCC, from 1985 to December 2000. The referral region of this department is the same as the area covered by the above-mentioned cancer registry. With permission of the patients involved, information on family history and on the results of MSI analysis, immunohistochemical and mutation analysis were used for this study. The medical ethics committees of the University Medical Center Groningen, Groningen, The Netherlands, and other participating hospitals approved the study.

Mutation analysis

Mutation analysis of the *MLH1, MSH2* and *MSH6* genes was carried out on DNA isolated from peripheral blood lymphocytes by denaturing gradient gel electrophoresis, followed, in the case of aberrant band patterns, by direct sequencing of independently amplified polymerase chain reaction products as described previously.[23] Validating homemade denaturing gradient gel electrophoresis systems for more than 40 different genes and testing hundreds of proved variants resulted in a sensitivity of the electrophoresis of almost 100% in our laboratory.[24] For the detection of large deletions (exonic deletions or deletions of a complete gene) and duplications, we used the *MLH1/MSH2* exon deletion multiplex ligation-dependent probe amplification test (MRC-Holland, Amsterdam, The Netherlands).[25] These data, for cases that had deletions of ⩾1 exon in the *MLH1* or *MSH2* gene, were confirmed by Southern blot analysis.[26]

For the purpose of this study, pathogenic mutations were defined as changes in the gene sequences that cause allele inactivation either because of the production of a truncated protein product or because of an exonic deletion or duplication. Initially, mutation analysis for the three genes was carried out in every patient, but in the last one third of patients, mutations in *MLH1* and *MSH2* were sought for only those with an MSI-high tumour. As a result, in 60 of the 281 patients, mutation analysis was carried out only in *MSH6*, and not in *MLH1* and *MSH2*.

Microsatellite instability analysis

MSI markers were used as previously defined, including two mononucleotide repeats (BAT25 and BAT26) and three dinucleotide repeats (D2S123, D5S346 and D17S250).[27] DNA was extracted from formalin-fixed, paraffin-wax-embedded tumour sections. For MSI analysis, control DNA was obtained from normal tissue from paraffin-wax-embedded sections or from peripheral blood lymphocytes from the same patient. MSI analysis was carried out as described previously.[21] Cancers were classified as ''MSI high'' when ⩾2 markers showed MSI and as ''MSI low'' when ⩽1 marker showed MSI. As a limited number of markers were analysed, the classification ''microsatellite stable'' was not used.

Immunohistochemical analysis

Immunohistochemistry for the MLH1, MSH2 and MSH6 proteins was carried out as described previously.[21] Protein expression in normal tissue adjacent to the cancer served as an internal positive control. The sections were scored as either negative (ie, absence of detectable nuclear staining of cancer cells) or positive for MLH1, MSH2 and MSH6 staining. The staining was scored blinded to the MSI or mutation status.

Predictive values of different strategies to select patients for mutation analysis

The variables family history and results of MSI and immunohistochemisty (IHC) were analysed and different selection strategies were investigated to determine the optimal way to select patients for mutation analysis. The positive and negative predictive values, sensitivity and specificity of each strategy, and 95% confidence intervals (CIs) were calculated. In addition, we calculated the same values for ''referral''; referral is defined as a patient having been referred to and counselled by a clinical geneticist versus inclusion within the framework of the study. For these calculations, the presence of a pathogenic germline mutation was the gold standard.

Statistical analysis

For statistical analysis, the χ^2 test and the two-sided t test were carried out and $p<0.05$ was considered to be significant. The independence of the variables family history, MSI, IHC and referral for detecting MMR gene mutations was examined using multivariate analysis with backward selection.

RESULTS

Clinical data

A total of 281 apparently unrelated patients were included. Table 1 summarises their clinical data. Table 2 shows the characteristics of their first-degree relatives with cancer. Families of 21 index patients fulfilled the revised ACII.[5]

Table 1 Patient characteristics

Characteristics	n	Age (years)
Total number	281	
Men:women	128:153	
Mean age (years) at diagnosis of first cancer (SD, range)		45 (11), 13–77
Men	128	46 (10), 19–75
Women	153	45 (11), 13–77
Patients referred to the study	212	46 (10), 20–77
Patients referred to the clinical geneticist	69	42 (11, 13–71
Patients with colorectal cancer at <50 years	224	
Patients with two HNPCC-associated cancers, including at least one CRC	79	
First cancer at <60 years	252	
Men:women	114:138	
One CRC	202	
Two cancers	60	
CRC 2×	34	
CRC/EC	14	
CRC/BLC	7	
CRC/DC	2	
CRC/STC	2	
CRC/PC	1	
Three cancers	13	
CRC 3×	7	
CRC 2×/STC	3	
CRC 2×/EC	2	
CRC 2×/BLC	1	
Four cancers	4	
CRC 4×	1	
CRC 3×/OC	1	
CRC 3×/EC	1	
CRC 2×/EC/BLC	1	
Eight cancers	2	
CRC 3×/EC/OC/UC 2×/RPC	1	
CRC 6×/EC/UC	1	

BLC, bladder cancer; CRC, colorectal cancer; DC, duodenal cancer; EC, endometrial cancer; HNPCC, hereditary non-polyposis colorectal cancer; OC, ovarian cancer; PC, pancreatic cancer; RPC, renal pelvic cancer; STC, stomach cancer; UC, ureteric cancer.

Table 2 Cancer diagnosis in first-degree relatives

Characteristics	Number	Age (years)
Patients with FD relative with HNPCC-associated cancer	111	
Mean age (SD, range) at diagnosis of first cancer of index patient		47 (11, 13–77)
Number of affected parents*	88 parents (2× both parents)†	
CRC	49	
EC	12	
STC	12	
GIC	4	
PC	2	
RPC, UC, BLC	10	
BTC	3	
OC	3	
Siblings with CRC	28 (including 1 half-sibling)	
Siblings with HNPCC-associated cancer other than CRC	14 (including 1 half-sibling)	
Children with CRC	3	
Children with HNPCC-associated cancer other than CRC	1	
Patients without an FD relative with HNPCC-associated cancer	170	
Mean age (SD, range) at diagnosis of first cancer of index patient		45 (10, 19–75)

BLC, bladder cancer; BTC, biliary tract cancer; CRC, colorectal cancer; EC, endometrial cancer; FD, first-degree; GIC, gastrointestinal cancer; HNPCC, hereditary non-polyposis colorectal cancer; OC, ovarian cancer; PC, pancreatic cancer; RPC, renal pelvic cancer; STC, stomach cancer; UC, ureteric cancer.
*Some parents had multiple HNPCC-associated tumours.
†Five patients had a parent with CRC and parent with an HNPCC-associated cancer.

However, not all cancers were histologically verified in 15 of these families.

Mutation analysis

A total of 18 different pathogenic mutations were identified in 25 patients (8 in *MLH1*, 9 in *MSH2* and 8 in *MSH6*; 25/281, 8.9%; table 3). All mutations were found in the 252 patients who had their first cancer diagnosed before the age of 60 years. Of the 17 *MLH1* and *MSH2* mutations, 15 occurred in patients who had their first cancer diagnosed before the age of 50 years, in contrast with four of the eight *MSH6* mutations. The mean age of the mutation carriers at the time of diagnosis of the first cancer was 42 (range 13–56) years.

Of the 25 mutation carriers, 19 had a first-degree relative with an HNPCC-associated cancer. Thus, 19 of 111 (17%) patients with a positive first-degree family history proved to have a mutation, versus 6 of 170 (3.5%) of the others ($p<0.001$). Only 9 of the 25 mutation carriers came from the 21 ACII-positive families. Thus, the sensitivity of the revised Amsterdam criteria in this study was 36% and the specificity was 95%. Among those referred for genetic counselling at the Department of Clinical Genetics, a larger proportion of patients proved to be mutation carriers than among those who were directly referred to the study (11/69, 16% *v* 14/212, 6.6%; $p = 0.018$). The mean age at diagnosis of CRC in relatives of mutation carriers was 47.4 years, whereas it was 63.2 years in relatives of index patients without a mutation ($p<0.001$). The mean age at diagnosis of endometrial cancer was not different between the two groups (49.2 *v* 54.5 years; $p = 0.138$).

Nineteen missense mutations of unknown pathogenicity (7 in *MLH1*, 4 in *MSH2* and 8 in *MSH6*) were detected in 18 patients. These missense mutations were not present in 200 healthy Dutch controls. One patient had an *MSH6* missense mutation combined with a pathogenic *MLH1* mutation, and another an *MLH1* missense combined with an *MSH6* missense mutation. For the missense mutation carriers, the mean age at onset of the first cancer was 45 (range 19–65) years. Seven of the 18 missense mutation carriers had a positive first-degree family history. As we do not know whether these missense mutations contribute to the disease phenotype, we excluded them from the study calculations. The clinical data on the missense mutation carriers are summarised in table A provided online at http://gutjnl.com/supplemental.

Microsatellite instability analysis

Tumour material was available for MSI analysis for 260 of the 281 patients. In 222 patients, only one cancer was available, whereas two cancers were available in 37 and three in one patient. Ninety seven patients had at least one MSI-high tumour. Among these patients, a higher frequency of mutation carriers was found than among the 163 patients with only MSI-low cancers (22/97 *v* 3/163, $p<0.001$). Three *MSH6* mutation carriers, remarkably all with the 649–650insT mutation, had only MSI-low cancers.

Table 3 Mismatch repair gene mutation carriers their cancer history and family history, and the results of immunohistochemistry and microsatellite instability analysis

Patient number, sex	Gene / exon	DNA change/ protein change	Cancer/ age at diagnosis (years)	MSI H/L	IHC: presence of			FH/ACII
					MLH1	MSH2	MSH6	
1. Male	*MLH1*	18del(	CRC/45	ND	ND	ND	ND	+/−
	1	GTTATTCGGCGGCTGGA)	CRC/49	H	−	+	ND	P: CRC 42
		Gly6FsX29	STC/53	L	ND	ND	ND	
2. Female	*MLH1*	c. 380G→A	CRC/13	H	−	+	+	+/−
	4	SD destroyed						P: CRC/27
3. Female	*MLH1*	c. 860_861insA	CRC/46	H	−	+	+	+/+
	10	p. Asn287fsX306	CRC/46	ND	ND	ND	ND	M: CRC/80
4. Female	*MLH1*	Del exon 16	CRC/50	H	−	+	+	+/+
	16		EC/50	H	ND	ND	ND	M: EC/50 S: CRC/48
5. Male	*MLH1*	Del exon 16	CRC/44	H	NI	+	+	−/−
	16							
6. Male	*MLH1*	Del exon 16	CRC/41	ND	ND	ND	ND	−/−
	16		CRC/48	ND	ND	ND	ND	
			CRC/48	H	+	+	+	
7. Female	*MLH1*	c. 1852–1854delGAA	CRC/25	H	−	+	ND	+/−
	16	p. Lys618del						P: CRC/36
8. Female*	*MLH1*	c. 1946delC	EC/49	H	−	+	NI	+/+
	17	p. Pro649fsX661	CRC/53	H	−	+	+	P: CRC/72 S: EC/?
9. Female	*MSH2*	Del exon 1–3	BLC/56	H	+	NI	−	−/−
	1–3		2xCRC/58	H	+	−	−	
			EC/59	L	+	−	−	
10. Male	*MSH2*	Del exon 2	CRC/46	H	ND	ND	ND	+/+
	2	Out of frame del						M: EC/59
11. Female	*MSH2*	Del exon 2	EC/42	H	+	−	−	+/+
	2	Out of frame del	CRC/47	H	+	−	−	M: EC/39
12. Female	*MSH2*	c. 759delG	OC/42	ND	ND	ND	ND	−/−
	4	p. Met253fsX273	CRC/48	H	+	−	ND	
			CRC/62	ND	ND	ND	ND	
			CRC/65	ND	ND	ND	ND	
13. Male	*MSH2*	Del exon 4–7	CRC/38	ND	ND	ND	ND	+/−
	4–7	Out of frame del	CRC/50	H	+	−	+	P: STC/43
14. Female	*MSH2*	Del exon 11–14	EC/48	H	+	−	−	+/+
	11–14	In frame deletion of 183 amino acids	CRC/54	H	+	−	−	M: EC/43
15. Female	*MSH2*	c. 1835C→G	CRC/31	ND	ND	ND	ND	+/+
	12	p. Ser612stop	CRC/53	H	+	−	NI	P: CRC/51
16. Female	*MSH2*	c. 1861C→T	CRC/29	H	+	−	ND	+/−
	12	p. Arg621stop						M: RPC/55
17. Male	*MSH2*	Del exon 12–16	CRC/25	H	+	−	−	+/+
	12–16	Out of frame deletion						M: EC/56
18. Female	*MSH6*	c. 649–650insT	CRC/59	L	+	+	−	+/−
	4	p. Asp217fsX218	EC/65	L	+	+	+	P: CRC/78 B: CRC/55
19. Female	*MSH6*	c. 649–650insT	DC/51	L	+	+	−	+/−
	4	p. Asp217fsX218	CRC/51	ND	ND	ND	ND	S: CRC/43
20. Male	*MSH6*	c. 649–650insT	CRC/41	L	+	+	−	+/−
	4	p. Asp217fsX218						M: BTC/82
21. Female	*MSH6*	c. 649–650insT	CRC/45	H	NI	NI	NI	+/−
	4	p. Asp217fsX21	CRC/53	H	NI	NI	NI	M: GIC/68 S: STC/46
		8	STC/62	ND	ND	ND	ND	S: EC/50
22. Female	*MSH6*	c. 649–650insT	CRC/50	ND	ND	ND	ND	+/+
	4	p. Asp217fsX218	CRC/83	H	+	+	−	SN: CRC/37 D: EC/55
23. Male	*MSH6*	c. 2672delT; 2674delT	CRC/55	H	−	+	−	+/−
	4	p. Ile891fsX899	CRC/55	H	−	+	+	B: CRC/42
24. Male	*MSH6*	c. 3262–3263insT	CRC/38	H	+	+	+	−/−
	5	p. Phe1088fsX1092						
25. Female	*MSH6*	c. 3772C→T	8 cancers†					−/−
	8	Gln1258sto p.	CRC/77	L	+	+	−	
			RPC/63	H	ND	ND	ND	

ACII, Amsterdam criteria II; BLC, bladder cancer; BTC, biliary tract cancer; C, DNA changes; CRC, colorectal cancer; D, daughter; DC, duodenal cancer; EC, endometrial cancer; FH, first-degree family history for HNPCC-associated tumours; GIC, gastrointestinal cancer; HNPCC, hereditary non-polyposis colorectal cancer; IHC, immunohistochemistry; M, mother; MSI, microsatellite instability; ND, not done; NI, not interpretable; OC, ovarian cancer; P, father; P, protein changes; RPC, renal pelvic cancer; S, sibling; SD, splicing donor site; SN, son; STC, stomach cancer; UC, ureteric cancer.
*This patient also carried an *MSH2* missense mutation.
†Other cancers in this patient were OC/49, CRC/55, EC/57, UC/65, UC/77 and CRC/77.

When more than one cancer per patient was analysed for MSI, a discrepancy between the MSI results occurred in 9 of 38 cases, including three mutation carriers (patients 1, 9 and 25; table 3).

Immunohistochemical analysis

Interpretable tumour specimens for MLH1 and MSH2 immunostaining were available from 209 patients, whereas those for MSH6 immunostaining were available from 183 patients. Absence of at least one of the MMR proteins was seen in tumours from 50 patients. Complete IHC results were available for 17 of the 25 mutation carriers (only these cases are included in calculation of the predictive values). In all 20 cases with staining absent for one of the MMR proteins, absence of the MMR proteins was concordant with the corresponding mutation. Two mutation carriers (MLH1, case

6; and MSH6, case 24) were missed when IHC would have been used as a selection criterion. In 4 of the 24 patients from whom multiple cancers could be analysed, discrepancy in the IHC results was found, including two *MSH6* mutation carriers (patients 18 and 23).

Predictive values of family history, MSI, IHC and referral for presence of a mutation

Table 4A shows the sensitivity, specificity and positive and negative predictive values of the different variables to predict a mutation. Calculations are based on data from the 174 patients from whom a complete set of variables was available. Similar calculations were made for MSI and IHC after preselection based on family history (table 4B) and for IHC after preselection based on MSI analysis (table 4C). The predictive values were also calculated for the three protein stainings separately (table 4D).

In the multivariate analysis with backward selection, a positive family history and negative staining of an MMR protein were found to be independent variables. The variables ''referral'' and MSI-high phenotype are not independent determinants. Absent staining of an MMR protein showed the highest odds ratio (table 4E).

DISCUSSION

This study was conducted primarily to define parameters that can reliably predict the presence of MMR gene mutations in two specific groups of patients with CRC suspected of having HNPCC—that is, patients with CRC before the age of 50 years and those with a CRC and at least one other HNPCC-associated cancer, irrespective of age. The main conclusions that can be drawn from this study are as follows. (1) Immunohistochemical staining for the MMR proteins MLH1, MSH2 and MSH6 is the best single method to select patients with CRC, suspected of having an MMR gene mutation, for mutation analysis. (2) The low prevalence of MMR gene mutations in patients with CRC below the age of 50 years justifies preselection by IHC and/or MSI analysis before mutation analysis is carried out. (3) The occurrence of ⩾2 HNPCC-associated cancers in patients aged ⩾60 years is rarely due to germline MMR gene mutations.

Recently, five large studies were published that also focused on the relative values of MSI analysis and IHC to detect potential germline MMR gene mutation carriers.[7–9 11 28] The conclusion from all five studies and from several previous studies was similar to that from ours—namely, IHC is at least equally valuable for this goal as MSI analysis. There are, however, several differences between our study and the other five.

In three of the studies, immunostaining for the MSH6 protein and mutation analysis for germline mutations in *MSH6* were not carried out.[8 9 11] Although less prevalent in typical AC-positive families, our results suggest that *MSH6* mutations occur at about the same rate as those in *MLH1* and *MSH2* in a less selected patient population. In the study by Hampel *et al*,[8] mutation analysis for *MSH6* was carried out only in patients whose tumours showed MSI (low or high) or who were considered to be at high risk for HNPCC on the basis of clinical criteria and whose tumours lacked MMR gene expression. Although, probably, most mutations were detected by this approach, some *MSH6* mutations could have been missed. Only Southey *et al*[7] carried out IHC for MSH6 in all tumours.

Stormorken *et al*[28] included only patients who were referred for genetic counselling. This may have influenced the resulting predictive values. The same is true for the sensitivity

Table 4 Predictive values

A: Predictive values (% and 95% CI) of the variables first-degree family history, MSI analysis, IHC analysis for the MLH1, MSH2 and MSH6 proteins and referral to clinical geneticist to detect germline mutations (n = 174)

	Sensitivity	Specificity	PPV	NPV
Family history	76% (53% to 90%)	64% (56% to 71%)	19% (11% to 29%)	96% (91% to 98%)
MSI analysis	82% (59% to 94%)	70% (62% to 77%)	23% (14% to 35%)	97% (92% to 99%)
IHC analysis	88% (66% to 97%)	84% (78% to 89%)	38% (24% to 53%)	99% (95% to 100%)
Referral	29% (13% to 53%)	90% (84% to 94%)	24% (11% to 45%)	92% (87% to 95%)

B: Predictive values (% and 95% CI) to detect germline mutations after preselection for a positive first-degree family history (n = 70)

	Sensitivity	Specificity	PPV	NPV
MSI analysis	59% (36% to 78%)	90% (85% to 94%)	40% (23% to 59%)	95% (91% to 98%)
IHC analysis	76% (53% to 90%)	95% (90% to 97%)	62% (41% to 79%)	97% (93% to 99%)

C: Predictive values (% and 95% CI) of IHC analysis to detect pathogenic mutations after preselection for MSI-high tumours (n = 61)

	Sensitivity	Specificity	PPV	NPV
IHC analysis	71% (47% to 87%)	89% (83% to 93%)	41% (26% to 59%)	97% (92% to 99%)

D: Predictive values (% and 95% CI) of immunohistochemical stainings of MLH1, MSH2 and MSH6 (n = 174)

	Sensitivity	Specificity	PPV	NPV
MLH1	80% (38% to 96%)	89% (84% to 93%)	18% (7.3% to 38%)	99% (96% to 100%)
MSH2	100% (57% to 100%)	96% (92% to 98%)	45% (21% to 72%)	100% (98% to 100%)
MSH6	86% (49% to 97%)	93% (88% to 96%)	33% (16% to 56%)	99% (96% to 100%)

E: Odds ratios for the presence of a germline mismatch repair gene mutation, calculated by multivariate analysis with backward selection for the variables first-degree family history, referral, MSI and IHC (n = 174)

	p Value	OR	95% CI
Family history	0.013	5.4	1.4 to 20.0
IHC	0	38.3	9.0 to 184
MSI	0.207	2.5	0.6 to 10.2
Referral	0.263	0.4	0.1 to 2.1

IHC, immunohistochemistry; MLH, MutL homolog; MSH, MutS homolog; MSI, microsatellite instability; NPV, negative predictive value; PPV, positive predictive value.

and the positive predictive value of the Amsterdam criteria, which can be expected to be high in a selected population like the one studied by Stormorken *et al*. We observed that only 9 of 25 mutation carrier families fulfilled the revised Amsterdam criteria. Also, Hampel *et al*[8] found only a small minority (3/23) of the mutation carriers' families fulfilling the Amsterdam criteria.

A limitation of many studies on this topic is that selection criteria are compared with each other, without establishing the gold standard—that is, the presence of mutations—in all participants. This is also true for four of the five studies mentioned above. Although the assumption that all pathogenic mutations of *MLH1* and *MSH2* lead to MSI can be well defended, a proper calculation of the sensitivity, specificity and predictive values requires a true gold standard. We carried out mutation analysis for all three genes in the first two thirds of included patients and did not find any clearly pathogenic mutation in *MLH1* or *MSH2* in patients with MSI-low tumours.

The value of IHC depends partially on the quality of the nuclear stainings and the experience of the pathologist.[12] The results are sometimes not interpretable because of absence or low intensity of the nuclear staining in tumour and normal cells.[29] Mangold *et al*[30] described the phenomenon of weak nuclear MLH1 staining in tumours of patients with *MLH1* mutations, which can be observed as false-positive staining. Experienced pathologists should be able to recognise these tumours with weakly positive MLH1 staining as tumours from possible *MLH1* mutation carriers. On the other hand, MSI analysis gives difficulties as well. The quality of tumour DNA isolated from paraffin-wax-embedded tumours may be poor and not usable for MSI analysis. Although seldom mentioned, interobserver variation also occurs when scoring MSI. About 15% of sporadic colorectal cancers show MSI and MLH1-negative stainings as a result of hypermethylation of the promoter region of *MLH1*.[31] Table 4D shows that MLH1 staining has a low positive predictive value. The consequence of this is that more patients will be screened for *MLH1* mutations with a negative outcome than is the case for the other two genes.

Apart from IHC and MSI analysis, we also evaluated the first-degree family history for HNPCC-associated cancers as an indicator of an MMR gene mutation. As expected, most mutation carriers had a first-degree family member with such a tumour. Nevertheless, 6 of the 25 (almost 25%) mutation carriers had a negative first-degree family history. This indicates that family history alone is an insufficient indicator of being a mutation carrier and we should not rely on it alone when deciding whether or not to carry out mutation analysis. We further calculated the predictive values for combinations of family history, MSI analysis and IHC. Although the specificity and positive predictive value both increased from this, the sensitivity decreased markedly. Therefore, when a patient fulfils one of the criteria used in this study, IHC or MSI analysis should be carried out without further selection. Both MSI and IHC analysis are proper selection tools, but when the family history raises high suspicion for HNPCC, both methods should be used to avoid missing mutation carriers.

Terdiman *et al*[32] noticed that referral for genetic counselling because of the suspicion of hereditary cancer was an independent risk factor for the presence of an MMR gene mutation when compared with population-based patients with CRC. We could not confirm that finding, although we also found a higher prevalence of mutations in patients referred to the clinical geneticist. A positive family history in most of those referred to a clinical geneticist is probably the determinant of this finding.

Our group of 224 patients with CRC at $<$50 years of age is the largest group so far reported in a single study. Most of these patients were referred specifically to participate in this study, whereas a minority had been referred to a clinical geneticist because of a perceived high risk for hereditary cancer. All patients came from the same geographical area and had their tumours diagnosed in approximately the same period. We therefore think that this group can be considered representative of all patients with CRC before the age of 50 years. Pathogenic mutations were detected in 14 of the 224 (6%) patients. *MLH1* and *MSH2* mutations were identified in 11 (5%) patients. This is similar to the findings of Pinol *et al*[11] and in accordance with prevalences of 0–12% in other small, otherwise unselected groups of such patients.[33–38] Owing to the low mutation frequency, it is, in our view, justified to carry out only mutation analysis in such patients if a screening by MSI analysis or IHC analysis suggests a mutation.

This may be different for people with $\geqslant$2 HNPCC-associated cancers, including at least one CRC. In this group of 79 people, we did not find any mutation in the 29 patients who had their first tumour diagnosed after age 60 years (19 of these had only CRCs and 10 had CRC and at least one other HNPCC-associated cancer). It is therefore questionable whether such patients should be screened for HNPCC and thus should be included in the Bethesda criteria. However, our group of patients was still rather small and further studies should be conducted before an age criterion for these patients can be confidently introduced. Nevertheless, when this subgroup is excluded from consideration, mutations were found in 17 of 50 patients with $\geqslant$2 cancers, of which at least one was diagnosed before age 60 years. Such a high occurrence may justify direct mutation analysis, without a prescreen for MSI or IHC.

Recently it was reported that the mean age at diagnosis of CRC of mutation-positive relatives of probands, identified in a population-based study of patients with CRC, was 61.2 years, much higher than that reported so far for mutation-positive people, which is about 45 years.[39] It is striking though, that the mean age at diagnosis of the probands in Hampel *et al*'s[39] study was 44 years. As patients in our study were collected irrespective of their family history, a similarly higher age at diagnosis of CRC in the relatives of our patients might have been expected. This was not the case as the mean age was 47.4 years. The fact that we did not find any mutation carriers in patients with $\geqslant$2 cancers, all diagnosed above age 60 years, also does not support the findings of Hampel *et al*[39]. Hence, their findings need confirmation in other populations, before any change in counselling practices is considered.

Only 8 of 25 supposedly mutation-carrying parents had CRC at the time of the study. Most of these 25 parents were older ($\geqslant$70 years) at that time and it was not expected that many of them would still develop CRC. This observation is in accordance with recent new calculations on the CRC risk in HNPCC—that is, 26.7% and 22.4% for men and women, respectively.[40]

A limitation of our study is the number of patients for whom a complete set of variables required for the calculations was available (n = 174). Although smaller than the populations in the studies by Engel *et al*[9] (n = 1119) and Pinol *et al*[11] (n = 287), we think that the number of patients in our study was large enough for reliable calculations. Another limitation might be the fact that we have missed mutations, as we included only the major HNPCC-related genes in our genetic screening. Mutations in other MMR genes, such as *PMS2*, cannot be ruled out.[41–45] Furthermore, we did not screen for mutations in the regulatory sequences (such as the promoter regions) of *MLH1*, *MSH2* and *MSH6*, nor did we look for genomic rearrangements in *MSH6*.[46 47] However, as such mutations probably represent only a small proportion of

disease-causing MMR gene mutations, this will not markedly alter the estimated prevalence of germline MMR gene mutations among patients with CRC. Finally, we cannot exclude survival bias in our study. Patients with MSI-high tumours have a better prognosis than those with MSI-low tumours, and, as we included some of the patients retrospectively and inclusion for the study required a blood sample for mutation analysis, the real mutation frequency might be somewhat lower.

The equal or even better potency of IHC to recognise patients with an MMR gene mutation does not apply to the detection of missense variants of currently unknown pathogenicity. In our study, 5 of 18 patients with such a variant had an MSI-high tumour, whereas IHC showed normal staining of the protein, corresponding with the variant gene, in all 14 cases where IHC was available (table A provided online). For some of the known missense variants, the evidence for pathogenicity is strong, but for most of them this is questionable. Functional assays of these variants may elucidate their pathogenic potential, but these have not yet been developed to a stage of routine application.

In conclusion, this study confirms that IHC staining for the MMR proteins is the best single method to select patients with CRC, suspected of having an MMR gene mutation, for mutation analysis. Such selection is mandatory for most patients with CRC before the age of 50 years, in view of the low prevalence of mutations in those patients. The value of genetic analysis in patients with multiple HNPCC-associated cancers, all occurring above age 60 years, is questionable.

ACKNOWLEDGEMENTS

We thank, in addition to the referring specialists in our hospital, the following specialists for patient referral: MA Alleman, JJ van Beek, JHW Bergmann, SB Bong, RWFR van den Broek, JG Bruyel, JW van Capelle, JLLM Coenen, GMJ DeKoninck, PFJ Donderwinkel, HH de Haan, LD de Haan, M van Haastert, H van der Heide, C Hoff, J Jager, HJA Jebbink, SS Klop, JJ Kolkman, WG Kootstra (deceased), L Meerman, HCJ van der Mijle, GF Nelis, M Oudkerk Pool, EGJM Pierik, W van Rooyen, CGBM Rupert, P Spoelstra, W Tel, JC Thijs, NP Tjon Pian Gi, JH Tomee, J Vecht, RJ Vermeer, RJ Vierhout, RA Weemhoff, EEM Weernink, EJGM Wichgers, HJ Wolters, LFA Wymenga and LS van der Zouwen. We thank J Schakenraad and M Schaapveld, Comprehensive Cancer Centre North-Netherlands, Groningen, The Netherlands, for selection of patients fulfilling our inclusion criteria from the regional cancer registry. We also thank RGJ Mensink and KA Kooi for their help with the mutation analysis, T van der Sluis and W Boersma for the immunohistochemical analysis and NJW Arts for her assistance in carrying out Southern blot analysis.

Authors' affiliations

R C Niessen*, Y Wu, R H Sijmons, H E K de Walle, C H C M Buys, R M W Hofstra, Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
M J W Berends*, J H Kleibeuker, Department of Gastroenterology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
H Hollema, A Karrenbeld, Department of Pathology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
M J L Ligtenberg, Departments of Human Genetics and Pathology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
E G E de Vries, Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
A G J van der Zee, Department of Gynaecology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

*RCN and MJWB contributed equally to this work.

Funding: This work was supported by grant numbers RUG 1997–1544 and RUG 2002–2678 from the Dutch Cancer Society.

Competing interests: None.

Ethical approval: The medical ethics committees of the University Medical Center Groningen, Groningen, The Netherlands, and other participating hospitals approved this study (reference number MEC 97/02/037).

REFERENCES

1 **Pedroni M**, Tamassia MG, Percesepe A, *et al*. Microsatellite instability in multiple colorectal tumors. *Int J Cancer* 1999;**81**:1–5.
2 **Lynch HT**, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;**348**:919–32.
3 **Abdel-Rahman WM**, Mecklin JP, Peltomaki P. The genetics of HNPCC: application to diagnosis and screening. *Crit Rev Oncol Hematol* 2006;**58**:208–20.
4 **Vasen HF**, Mecklin JP, Khan PM, *et al*. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum* 1991;**34**:424–5.
5 **Vasen HF**, Watson P, Mecklin JP, *et al*. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology* 1999;**116**:1453–6.
6 **Debniak T**, Kurzawski G, Gorski B, *et al*. Value of pedigree/clinical data, immunohistochemistry and microsatellite instability analyses in reducing the cost of determining hMLH1 and hMSH2 gene mutations in patients with colorectal cancer. *Eur J Cancer* 2000;**36**:49–54.
7 **Southey MC**, Jenkins MA, Mead L, *et al*. Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. *J Clin Oncol* 2005;**23**:6524–32.
8 **Hampel H**, Frankel WL, Martin E, *et al*. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 2005;**352**:1851–60.
9 **Engel C**, Forberg J, Holinski-Feder E, *et al*. Novel strategy for optimal sequential application of clinical criteria, immunohistochemistry and microsatellite analysis in the diagnosis of hereditary nonpolyposis colorectal cancer. *Int J Cancer* 2006;**118**:115–22.
10 **Lindor NM**, Burgart LJ, Leontovich O, *et al*. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol* 2002;**20**:1043–8.
11 **Pinol V**, Castells A, Andreu M, *et al*. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. *JAMA* 2005;**293**:1986–94.
12 **Müller W**, Burgart LJ, Krause-Paulus R, *et al*. The reliability of immunohistochemistry as a prescreening method for the diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC)—results of an international collaborative study. *Familial Cancer* 2001;**1**:87–92.
13 **Syngal S**, Fox EA, Eng C, *et al*. Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1. *J Med Genet* 2000;**37**:641–5.
14 **Wahlberg SS**, Schmeits J, Thomas G, *et al*. Evaluation of microsatellite instability and immunohistochemistry for the prediction of germ-line MSH2 and MLH1 mutations in hereditary nonpolyposis colon cancer families. *Cancer Res* 2002;**62**:3485–92.
15 **Christensen M**, Katballe N, Wikman F, *et al*. Antibody-based screening for hereditary nonpolyposis colorectal carcinoma compared with microsatellite analysis and sequencing. *Cancer* 2002;**95**:2422–30.
16 **Stormorken AT**, Müller W, Lemkemeyer B, *et al*. Prediction of the outcome of genetic testing in HNPCC kindreds using the revised Amsterdam criteria and immunohistochemistry. *Fam Cancer* 2001;**1**:169–73.
17 **Rodriguez-Bigas MA**, Boland CR, Hamilton SR, *et al*. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst* 1997;**89**:1758–62.
18 **Umar A**, Boland CR, Terdiman JP, *et al*. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004;**96**:261–8.
19 **Katballe N**, Juul S, Christensen M, *et al*. Patient accuracy of reporting on hereditary non-polyposis colorectal cancer-related malignancy in family members. *Br J Surg* 2001;**88**:1228–33.
20 **Sijmons RH**, Boonstra AE, Reefhuis J, *et al*. Accuracy of family history of cancer: clinical genetic implications. *Eur J Hum Genet* 2000;**8**:181–6.
21 **Berends MJ**, Wu Y, Sijmons RH, *et al*. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. *Am J Hum Genet* 2002;**70**:26–37.
22 **Kolodner RD**, Tytell JD, Schmeits JL, *et al*. Germ-line msh6 mutations in colorectal cancer families. *Cancer Res* 1999;**59**:5068–74.
23 **Wu, Y**. Design and application of DGGE-based mutation detection systems for genes involved in HNPCC [dissertation]. Groningen: University of Groningen, 1999. http://irs.ub.rug.nl/ppn/182343367 (accessed 23 Sept. 2006).
24 **Van der Hout AH**, Van den Ouweland AM, Van der Luijt RB, *et al*. A DGGE system for comprehensive mutation screening of *BRCA1* and *BRCA2*. Application in a Dutch cancer clinic setting. *Hum Mutat* 2006;**27**:654–66.
25 **Gille JJ**, Hogervorst FB, Pals G, *et al*. Genomic deletions of MSH2 and MLH1 in colorectal cancer families detected by a novel mutation detection approach. *Br J Cancer* 2002;**87**:892–7.

26 **Wijnen J**, Van der Klift H, Vasen H, *et al.* MSH2 genomic deletions are a frequent cause of HNPCC. *Nat Genet* 1998;**20**:326–8.
27 **Boland CR**, Thibodeau SN, Hamilton SR, *et al.* A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;**58**:5248–57.
28 **Stormorken AT**, Bowitz-Lothe IM, Noren T, *et al.* Immunohistochemistry identifies carriers of mismatch repair gene defects causing hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2005;**23**:4705–12.
29 **Chapusot C**, Martin L, Puig PL, *et al.* What is the best way to assess microsatellite instability status in colorectal cancer? Study on a population base of 462 colorectal cancers. *Am J Surg Pathol* 2004;**28**:1553–9.
30 **Mangold E**, Pagenstecher C, Friedl W, *et al.* Tumours from MSH2 mutation carriers show loss of MSH2 expression but many tumours from MLH1 mutation carriers exhibit weak positive MLH1 staining. *J Pathol* 2005;**207**:385–95.
31 **Thibodeau SN**, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993;**260**:816–19.
32 **Terdiman JP**, Levin TR, Allen BA, *et al.* Hereditary nonpolyposis colorectal cancer in young colorectal cancer patients: high-risk clinic versus population-based registry. *Gastroenterology* 2002;**122**:940–7.
33 **Aaltonen LA**, Salovaara R, Kristo P, *et al.* Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998;**338**:1481–7.
34 **Wang Q**, Lasset C, Desseigne F, *et al.* Prevalence of germline mutations of hMLH1, hMSH2, hPMS1, hPMS2, and hMSH6 genes in 75 French kindreds with nonpolyposis colorectal cancer. *Hum Genet* 1999;**105**:79–85.
35 **Dieumegard B**, Grandjouan S, Sabourin JC, *et al.* Extensive molecular screening for hereditary non-polyposis colorectal cancer. *Br J Cancer* 2000;**82**:871–80.
36 **Montera M**, Resta N, Simone C, *et al.* Mutational germline analysis of hMSH2 and hMLH1 genes in early onset colorectal cancer patients. *J Med Genet* 2000;**37**:E7.
37 **Pucciarelli S**, Agostini M, Viel A, *et al.* Early-age-at-onset colorectal cancer and microsatellite instability as markers of hereditary nonpolyposis colorectal cancer. *Dis Colon Rectum* 2003;**46**:305–12.
38 **Salovaara R**, Loukola A, Kristo P, *et al.* Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2000;**18**:2193–200.
39 **Hampel H**, Stephens JA, Pukkala E, *et al.* Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. *Gastroenterology* 2005;**129**:415–21.
40 **Quehenberger F**, Vasen HF, van Houwelingen HC. Risk of colorectal and endometrial cancer for carriers of mutations of the hMLH1 and hMSH2 gene: correction for ascertainment. *J Med Genet* 2005;**42**:491–6.
41 **Nakagawa H**, Lockman JC, Frankel WL, *et al.* Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein, but paralogous genes obscure mutation detection and interpretation. *Cancer Res* 2004;**64**:4721–7.
42 **Truninger K**, Menigatti M, Luz J, *et al.* Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. *Gastroenterology* 2005;**128**:1160–71.
43 **Worthley DL**, Walsh MD, Barker M, *et al.* Familial mutations in PMS2 can cause autosomal dominant hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2005;**128**:1431–6.
44 **Thompson E**, Meldrum CJ, Crooks R, *et al.* Hereditary non-polyposis colorectal cancer and the role of hPMS2 and hEXO1 mutations. *Clin Genet* 2004;**65**:215–25.
45 **De Jong AE**, van Puijenbroek M, Hendriks Y, *et al.* Microsatellite instability, immunohistochemistry, and additional PMS2 staining in suspected hereditary nonpolyposis colorectal cancer. *Clin Cancer Res* 2004;**10**:972–80.
46 **Plaschke J**, Ruschoff J, Schackert HK. Genomic rearrangements of hMSH6 contribute to the genetic predisposition in suspected hereditary non-polyposis colorectal cancer syndrome. *J Med Genet* 2003;**40**:597–600.
47 **Shin KH**, Shin JH, Kim JH, *et al.* Mutational analysis of promoters of mismatch repair genes hMSH2 and hMLH1 in hereditary nonpolyposis colorectal cancer and early onset colorectal cancer patients: identification of three novel germ-line mutations in promoter of the hMSH2 gene. *Cancer Res* 2002;**62**:38–42.

EDITOR'S QUIZ: GI SNAPSHOT

Answer

From question on page 1780

The gastrograffin enema shows leakage at the level of the splenic flexure of the colon.(fig. 1) The computed tomography scan shows a 10×8 cm well-encapsulated hypodense multicystic mass of the tail of the pancreas containing gas (fig 2).

Splenopancreatectomy with en bloc resection was carried out on the left colon and part of the stomach. A terminal right transverse colostomy was also performed. Histopathological examination confirmed the presence of a mucinous cystadenoma of the pancreas in contact with the colon, with no signs of malignancy.

The patient was admitted, 3 months later, to restore intestinal continuity. Local recurrence or distant metastases was not evident. She was seen 7 years later in apparently good health; abdominal computed tomography scan was normal.

Cystic neoplasms of the pancreas are acquired tumours. We distinguish the serous cystadenoma, also known as microcystic adenoma, which is usually benign, from the mucinous cystic neoplasms which encompass a spectrum that ranges from benign but potentially malignant lesions to carcinoma with a very aggressive behaviour. Complications arise owing to either vascular or visceral compression, or biliary and pancreatic fistulas. These lesions can be easily misidentified as benign cysts such as pseudocysts or simple pancreatic cysts and are often mismanaged as a pseudocyst.

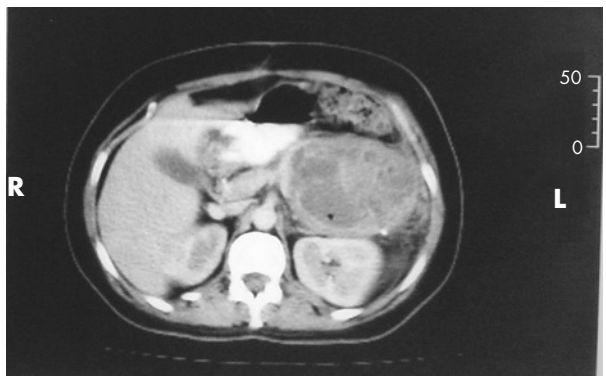

Figure 1 Computed tomography scan of the upper abdomen showing a 10×8 cm well-encapsulated hypodense multicystic mass of the tail of the pancreas containing gas.

This is the first case of a pancreatic mucinous cystadenoma with a fistula in the left colon described in the literature.

doi: 10.1136/gut.2005.090266